Report on the Determination of Total Nitrogen in Tobacco *

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The time-honored Kjeldahl method for the determination of nitrogen has been under continuous study and revision since its inception in 1883. Recent studies and action by the Association of Official Agricultural Chemists (*This Journal,* **39**, 63 (1956)) have resulted in the adoption of a single official method in which mercury or its oxide is the only catalyst. Potassium sulfate (or anhydrous sodium sulfate) is added to increase the digestion temperature and thus reduce the digestion time required, particularly for refractory compounds such as the alkaloids present in tobacco.

The Committee decided that the A.O.A.C. method should be tested to determine its applicability to the analysis of tobacco. A detailed copy of the procedure was sent to each collaborator. The only deviations from the official procedure as modified by Davis and Miles (*This Journal,* **39**, 550 (1956)) were:

1. The amount of acid specified was related to the sample weight (35, 37.5, 40 ml for 1, 1.5, 2 g nitrate-containing samples, and 20, 22.5, 25 ml for 1, 1.5, 2 g nitrate-free samples, respectively).

2. The mixed indicator methyl red-methylene blue could be used instead of methyl red.

3. The digestion time was one hour after clearing.

4. The analysts were given the option of using boric acid or standard acid for absorbing the ammonia.

Each collaborator was asked to analyze five tobacco samples in duplicate and report all values obtained on the "as received" basis. The samples were one each of burley, flue-cured, cigar filler, Maryland, and Turkish tobacco which had been ground to pass a 1 ml sieve, equilibrated in air, mixed, and sealed in screw-cap glass jars. Refrigerator tape was placed around the rim of the jar caps to reduce the possibility of change in moisture content between the time of bottling and analysis.

In addition to analyzing the samples by the A.O.A.C. method, the collaborators were asked to determine the total nitrogen in the samples by the procedure they normally used in their laboratory, to report the results of duplicate analyses, and to submit a detailed description of the procedure used. This was done to permit comparison of the results by the A.O.A.C. method with those obtained by different procedures which the collaborators found suitable for the analysis of tobacco.

METHOD

Reagents

See 2.21 and:

(a) *Sodium hydroxide-thiosulfate soln.*—Dissolve 500 g NaOH pellets and 40 g $Na_2S_2O_3 \cdot 5H_2O$ in H_2O and dil. to 1 L.

(b) *Methyl red indicator.*—Dissolve 1 g in 200 ml EtOH; *or* mixed indicator prepd by dissolving 0.8 g Me red and 0.2 g methylene blue in 500 ml EtOH.

Apparatus

See 2.22 as revised, *This Journal,* **39**, 81 (1956).

Kjeldahl Method for Nitrate-Containing Samples

(For nitrate-free samples omit salicylic acid and thiosulfate treatment.)

Place weighed sample (1–2 g) in digestion flask. Add vol. H_2SO_4 (contg 2 g salicylic acid/40 ml) corresponding to wt of sample (35 ml for 1 g, 40 ml for 2 g for NO_3-contg samples; 20 and 25 ml, resp., for NO_3-free samples). Shake until thoroly mixed and let stand 30 min. or more with occasional shaking; then add

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5 g $Na_2S_2O_3.5H_2O$. Shake, let stand 5 min., and heat carefully until frothing ceases. Turn off heat, add 0.7 g HgO (or metallic Hg) and 15 g K_2SO_4, and boil briskly 1–1.5 hr after soln clears.

Cool, add ca 200 ml H_2O, cool to ca room temp., and add few Zn granules. Tilt flask and carefully add 50 ml NaOH-thiosulfate soln without agitation. Immediately connect flask to distn bulb on condenser. Place 50 ml std 0.1*N* acid in receiving flask and place so that condenser tube is immersed in acid soln. Then rotate digestion flask carefully to mix contents. Heat until at least 150 ml distillate has been collected, and titr. excess acid with std base, using Me red or mixed indicator. Correct for blank detn on reagents.

Notes

1. Heaters should be adjusted to bring 250 ml H_2O from 25°C to a rolling boil in 4–6 minutes. To test heater, preheat for 10 minutes if gas, or 30 minutes if electric, and add 3 or 4 boiling chips to flask to prevent superheating.

2. 25 ml saturated H_3BO_3 may be used in place of standard 0.1*N* HCl and the ammonia titrated with standard 0.1*N* HCl (or H_2SO_4), using mixed indicator.

3. For nitrate-free samples, use volume of acid as follows: 1 g, 20 ml; 1.5 g, 22.5 ml; 2 g, 25 ml. Omit salicylic acid and thiosulfate treatment.

Results and Recommendation

The results obtained by the ten collaborators who used the A.O.A.C. procedure are shown in Table 1. Each value shown is an average of duplicate determinations. The intralaboratory standard deviation for each collaborator (s) was calculated from the difference between duplicates for the five sets of analyses by $s = \sqrt{\frac{(\text{sum of } d^2)}{2n}}$ where d is the difference between duplicates and n is the number of pairs. $\bar{x}$ is the mean of the collaborator's averages and s_m the interlaboratory standard deviation for each sample. The intra- and interlaboratory precision are good in all cases. The close agreement between the s_m values for the five samples indicates that neither the type of tobacco nor the nitrate content has much influence on the interlaboratory precision. The coefficients of variation for the five samples varied from 1.2 to 2.6%, which is considered good for samples with as low as 1.5% nitrogen.

Of the ten collaborators, five chose to use a standard acid and five saturated boric acid to absorb the ammonia evolved. The average per cent nitrogen obtained for all five samples was slightly higher for those who used the standard acid. However, no statistically significant differences were found. The slightly higher results with standard acid seem to be in agreement with previous A.O.A.C. studies comparing the two ammonia absorbents. Apparently, boric acid is a suitable absorbent for micro analysis but not for macro. It may be that the concentration of the ammonia in the initial phase of the distillation is sufficiently greater in the macro determination to lead to some loss.

The results obtained when the collaborators used their own methods and some of the details of the procedures are shown in Table 2. The intra- and interlaboratory standard deviations, s and s_m values, are, on the average, a little higher than those for the A.O.A.C. method. Only two collaborators (No. 4 and 11) failed to obtain slightly better precision with the A.O.A.C. method than with their own. The $\bar{x}$ values for the A.O.A.C. method for all five samples were slightly higher than those obtained when the collaborators used their own procedures.

An attempt was made to evaluate the collaborators' methods by ranking each method for each sample. The assumption was made that the highest value was the most nearly correct because it is known that for materials containing refractory compounds, the Kjeldahl nitrogen results tend to be low. By assigning a value of 1 to the method producing the highest value, 2 to the next highest, etc., and summing the rankings for the five samples, the five top methods in order were those of Collaborator 5, 11, 13, 12, and 9. The relative rankings were 6, 15, 16, 20, and 26. It is interesting to note that the method of Collaborator 5 differed from the A.O.A.C. method only slightly. Copper and mercury catalysts were used instead of only mercury, and about half as much potassium sulfate was added, but the digestion time was doubled.

Table 1. Percentages of total nitrogen found by the AOAC method[a]

Coll.	% Nitrogen in Sample 1	2	3	4	5	s	Acid Absorbent Standard	Acid Absorbent Boric
0[b]	3.52	1.57	4.60	2.20	1.80	0.023		x
2	3.50	1.62	4.52	2.23	1.82	0.015	x	
3	3.40	1.53	4.53	2.16	1.73	0.046		x
4	3.35	1.53	4.47	2.06	1.71	0.021		x
5	3.47	1.59	4.60	2.19	1.76	0.007	x	
7	3.30	1.49	4.48	2.15	1.69	0.015		x
9	3.46	1.56	4.51	2.14	1.75	0.028	x	
11	3.40	1.52	4.45	2.07	1.73	0.050	x	
13	3.40	1.53	4.46	2.14	1.68	0.021		x
14	3.36	1.54	4.46	2.12	1.69	0.030	x	
$\bar{x}$	3.42	1.55	4.51	2.15	1.74	(0.026)		
s_m	0.070	0.036	0.055	0.054	0.045			

[a] Each value is the average of duplicates.
[b] One-tenth specified amounts of sample and reagents used.

Table 2. Collaborative results for per cent total nitrogen[a] found and details of collaborators' procedures

Coll.	% Total Nitrogen in Sample 1	2	3	4	5	s	Sample Wt, g	Digestion[b] hr	H_2SO_4, ml	K_2SO_4, g	Catalyst	Reducing Agent	Trapping Acid
2	3.25	1.53	4.28	2.06	1.70	0.103	1.4	½	30	7–8	Se	Salicyclic acid	HCl
4	3.32	1.50	4.59	2.10	1.74	0.018	0.04	2	2	1	Cu–Hg	None	H_3BO_3
5	3.50	1.60	4.61	2.21	1.78	0.010	1.4	2	35	5	Cu–Hg	Salicyclic acid	H_2SO_4
7	3.27	1.50	4.43	2.09	1.68	0.037	0.5	1	25	15	Hg	Salicyclic acid	H_3BO_3
9	3.41	1.54	4.39	2.12	1.73	0.034	2	12	55	0	Hg–Se	Fe and Salicyclic acid	HCl
11	3.48	1.55	4.56	2.20	1.75	0.012	1	1–1½	40	10	HgO	Salicylic acid	HCl
12	3.49	1.57	4.55	2.16	1.71	0.037	1	12	40	0	Se	Fe and Salicylic acid	HCl
13	3.54	1.52	4.58	2.18	1.74	0.026	1–2	1	40	14	Cu	Salicylic acid	H_3BO_3
14	3.29	1.46	4.22	2.04	1.64	0.066	0.1	2	6.5	2.5	Se	Fe	H_3BO_3
$\bar{x}$	3.39	1.53	4.47	2.13	1.72	(0.038)							
s_m	0.113	0.041	0.145	0.061	0.042								

[a] Each value is the average of duplicates.
[b] Digestion time after clearing.

Table 3. Summary of means, standard deviations, and coefficients of variation for the two methods and five tobacco samples

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
			Means		
A.O.A.C.	3.42	1.55	4.51	2.15	1.74
Collaborator	3.39	1.53	4.47	2.13	1.72
			Standard Deviations		
A.O.A.C.	0.070	0.036	0.055	0.054	0.045
Collaborator	0.113	0.041	0.145	0.061	0.042
			Coefficients of Variation		
A.O.A.C.	2.05	2.32	1.22	2.51	2.59
Collaborator	3.33	2.68	3.24	2.86	2.44

The data from this study are summarized in Table 3. It would appear (*1*) that the A.O.A.C. method is satisfactory for the determination of total nitrogen in tobacco; (*2*) that standard acid rather than boric acid should be used for best results; and (*3*) that the digestion time should be increased to 1.5 hours to allow for the possibility that some types of digestion apparatus do not provide the optimum amount of heat to the digestion flask.

It is recommended* that the method be adopted as first action.

* For report of Subcommittee A and action of the Association, see *This Journal*, **42**, 18 (1959).

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